SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

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I. GENERAL INFORMATION

Device Generic Name:	filler, recombinant human bone morphogenetic protein, collagen scaffold, osteoinduction
Device Trade Name:	INFUSE® Bone Graft
Applicant's Name and Address:	Wyeth Pharmaceuticals, Inc. P.O. Box 8299 Philadelphia, Pennsylvania 19101-8299
Premarket Approval Application (PMA) Number:	P000054
Date of Panel Recommendation:	November 21, 2002
Date of Notice of Approval to Applicant:	April 30, 2004

II. INDICATIONS FOR USE

INFUSE Bone Graft is indicated for treating acute, open tibial shaft fractures that have been stabilized with IM nail fixation after appropriate wound management. INFUSE Bone Graft must be applied within 14 days after the initial fracture. Prospective patients should be skeletally mature.

III. CONTRAINDICATIONS

- INFUSE Bone Graft is contraindicated for patients with a known hypersensitivity to recombinant human Bone Morphogenetic Protein-2, bovine Type I collagen or to other components of the formulation.
- INFUSE Bone Graft should not be used in the vicinity of a resected or extant tumor, in patients with any active malignancy or patients undergoing treatment for a malignancy.
- INFUSE Bone Graft should not be used in patients who are skeletally immature (<18 years of age or no radiographic evidence of epiphyseal closure).
- INFUSE Bone Graft should not be used in patients with an inadequate neurovascular status, *e.g.*, high risk of amputation.

- INFUSE Bone Graft should not be used in patients with compartment syndrome of the affected limb.

- INFUSE Bone Graft should not be used in pregnant women. The potential effects of rhBMP-2 on the human fetus have not been evaluated.

- INFUSE Bone Graft should not be implanted in patients with an active infection at the operative site.

IV. WARNINGS AND PRECAUTIONS

WARNINGS:

- Women of childbearing potential should be advised that antibody formation to rhBMP-2 or its influence on fetal development have not been assessed. In the clinical trial supporting the safety and effectiveness of INFUSE Bone Graft in tibial fracture, 9/149 (6.0%) patients treated with INFUSE Bone Graft and 1/150 (0.7%) patients treated without exposure to rhBMP-2 developed antibodies to rhBMP-2. The effect of maternal antibodies to rhBMP-2, as might be present for several months following device implantation, on the unborn fetus is unknown. Additionally, it is unknown whether fetal expression of BMP-2 could re-expose mothers who were previously antibody positive, thereby eliciting a more powerful immune response to BMP-2 with adverse consequences for the fetus. Studies in genetically altered mice indicate that BMP-2 is critical to fetal development and that lack of BMP-2 activity, as might be induced by antibody formation, may cause neonatal death or birth defects.

- The safety and effectiveness of INFUSE Bone Graft in nursing mothers has not been established. It is not known if BMP-2 is excreted in human milk.

- Women of childbearing potential should be advised not to become pregnant for one year following treatment with INFUSE Bone Graft.

Please refer to the device labeling for a complete list of warnings and precautions.

V. DEVICE DESCRIPTION

INFUSE Bone Graft consists of two components – a recombinant human bone morphogenetic protein solution and a carrier/scaffold for the bone morphogenetic protein solution and resulting bone. These components must be used as a system. The bone morphogenetic protein solution component must not be used without carrier/scaffold component or with a carrier/scaffold component different from the one described in this document.

INFUSE Bone Graft consists of recombinant human Bone Morphogenetic Protein-2 (rhBMP-2, known as dibotermin alfa) placed on an absorbable collagen sponge (ACS). INFUSE Bone Graft induces new bone tissue at the site of implantation. Based on data from non-clinical studies, the bone formation process develops from the outside of the implant towards the center until the entire device is replaced by trabecular bone.

rhBMP-2 is the active agent in INFUSE Bone Graft. rhBMP-2 is a disulfide-linked dimeric protein molecule with two major subunit species of 114 and 131 amino acids. Each subunit is glycosylated at one site with high-mannose-type glycans. rhBMP-2 is produced by a genetically engineered Chinese hamster ovary cell line.

rhBMP-2 and excipients are lyophilized. Upon reconstitution, each milliliter of rhBMP-2 solution contains: 1.5 mg of rhBMP-2; 5.0 mg sucrose, NF; 25 mg glycine, USP; 3.7 mg L-glutamic acid, FCC; 0.1 mg sodium chloride, USP; 0.1 mg polysorbate 80, NF; and 1.0 mL of sterile water. The reconstituted rhBMP-2 solution has a pH of 4.5, and is clear, colorless and essentially free from plainly visible particulate matter.

The ACS is a soft, white, pliable, absorbent implantable matrix for rhBMP-2. ACS is made from bovine Type I collagen obtained from the deep flexor (Achilles) tendon. The ACS acts as a carrier for the rhBMP-2 and acts as a scaffold for new bone formation.

Each kit contains all the components necessary to prepare INFUSE Bone Graft: the rhBMP-2 which must be reconstituted, sterile water, absorbable collagen sponges, syringes with needles, this package insert and instructions for preparation.

The rhBMP-2 is provided as a lyophilized powder in vials delivering 12 mg of protein. After appropriate reconstitution, the concentration of rhBMP-2 is 1.5 mg/mL. The solution is then applied to the provided absorbable collagen sponge. INFUSE Bone Graft is prepared at the time of surgery and allowed a prescribed amount of time (no less than 15 minutes) before placement at the fracture site. The Instructions for Preparation contain complete details on preparation of INFUSE Bone Graft

VI. ALTERNATIVE PRACTICES AND PROCEDURES

Surgical alternatives to the use of INFUSE Bone Graft with an IM nail are related solely to the choice of stabilization method, *e.g.*, external fixator or plates and screws. There are no non-surgical alternatives to the treatment of acute, open tibial shaft fractures.

VII. MARKETING HISTORY

INFUSE Bone Graft has been marketed in the European Union since July, 2003 for a use similar to that described in the PMA. INFUSE Bone Graft has not been withdrawn from marketing for any reason relating to the safety and effectiveness of the device.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

The table below describes the adverse events observed in the clinical trial used to support approval of the product. Two INFUSE Bone Graft doses, 0.75mg/ml and 1.5mg/ml, were evaluated. INFUSE Bone Graft with IM nail stabilization was implanted in 300 investigational patients (149 in the 1.50 mg/ml and 151 in the 0.75 mg/ml groups) compared to IM stabilization alone in 150 control patients. Adverse event rates presented are based on the number of patients having at least one occurrence for a particular adverse event divided by the total number of patients in that treatment group. Additional adverse event information is presented in Section X.2.8.

Adverse events reported for patients enrolled in clinical trial supporting approval

	1st quarter postop			2nd quarter postop			3rd quarter postop			4th quarter postop			# (%) of Patients Total Adverse Events								
	Control	low dose	Inves.	Control	low dose	Inves.	Control	low dose	Inves	Control	low dose	Inves.	Control (n=150)		(%)	low dose (n=151)		(%)	Inves (n=149)		(%)
Number of patients	150	150	149	147	144	146	144	144	144	138	142	141	150		(100)	150		(99)	149		(100)
Abnormal healing																					
surgical site	64	60	53	19	19	13	7	4	7	4	2	3	65[a]	94[c]	(43)[b]	62	85	(41)	57	76	(38)
other locations	9	13	23	0	1	0	1	0	1	0	0	2	10	10	(7)	10	14	(7)	13	26	(9)
Abnormal lab tests																					
Alkaline phosphatase increased	8	8	3	0	0	0	0	0	0	0	0	0	8	8	(5)	8	8	(5)	3	3	(2)
Amylase increased	5	23	11	0	0	0	0	0	0	0	0	0	5	5	(3)	20	23	(13)	10	11	(7)
Bilirubinemia	8	9	11	0	0	0	0	0	0	0	0	0	7	8	(5)	9	9	(6)	11	11	(7)
BUN increased	0	3	1	0	0	0	0	0	0	0	0	0	0	0	(0)	3	3	(2)	1	1	(1)
Creatinine clearance decreased	2	1	2	0	0	0	0	0	0	0	0	0	2	2	(1)	1	1	(1)	2	2	(1)
Gamma glutamyl transpeptidase increased	0	1	2	0	0	0	0	0	0	0	0	0	0	0	(0)	1	1	(1)	2	2	(1)
Hypercalcemia	2	1	0	0	0	0	0	0	0	0	0	0	2	2	(1)	1	1	(1)	0	0	(0)
Hyperkalemia	2	3	3	0	0	0	0	0	0	0	0	0	2	2	(1)	3	3	(2)	3	3	(2)
Hypokalemia	7	9	17	1	0	0	0	0	0	1	0	0	9	9	(6)	9	9	(6)	17	17	(11)
Hypomagnesemia	3	11	3	0	0	0	0	0	0	0	0	0	2	3	(1)	11	11	(7)	3	3	(2)
Hypocalcemia	54	63	55	0	0	0	0	0	0	0	0	0	50	54	(33)	60	63	(40)	53	55	(36)
Lactic dehydrogenase increased	30	34	32	0	1	0	0	0	0	0	0	0	29	30	(19)	33	35	(22)	26	32	(17)
SGOT increased	43	47	42		1	0	0	0	0	0	0	0	39	43	(26)	46	48	(30)	40	42	(27)
SGPT increased	26	28	23	0	0	0	0	0	0	0	0	0	24	26	(16)	26	28	(17)	21	23	(14)
Other	134	119	117	0	0	0	0	1	0	0	0	0	80	134	(53)	82	120	(54)	77	117	(52)
Accidental injuries	1	2	4	1	0	2	0	0	1	0	0	2	2	2	(1)	2	2	(1)	6	9	(4)
Cardiovascular altered function	3	0	2	0	0	0	0	0	0	0	0	2	3		(2)	0		(0)	4		(3)

[a] Number of patients with event
[b] Percent of patients with event
[c] Total number of events

Adverse events reported for patients enrolled in clinical trial supporting approval

	1st quarter postop			2nd quarter postop			3rd quarter postop			4th quarter postop			# (%) of Patients Total Adverse Events								
	Control	low dose	Inves.	Control	low dose	Inves.	Control	low dose	Inves.	Control	low dose	Inves.	Control (n=150)		(%)	low dose (n=151)		(%)	Inves. (n=149)		(%)
														3			0			4	
altered rhythm	3	10	19	2	1	1	0	0	1	2	0	0	6	7	(4)	11	11	(7)	10	21	(7)
hypercoagulability, surgical site	2	2	1	0	0	0	0	0	0	0	0	0	2	2	(1)	2	2	(1)	1	1	(1)
hypercoagulability, general	3	5	2	0	0	1	0	0	0	0	0	0	3	3	(2)	4	5	(3)	3	3	(2)
hypertension/hypotension	7	10	6	2	2	0	0	1	0	2	0	0	8	11	(5)	12	13	(8)	6	6	(4)
other, surgical site	1	3	2	0	0	0	0	0	1	0	0	0	1	1	(1)	3	3	(2)	3	3	(2)
other, general	1	1	8	0	1	1	0	0	0	0	0	0	1	1	(1)	2	2	(1)	5	9	(3)
Digestive system																					
altered function	11	5	9	1	1	2	0	1	0	0	0	0	10	12	(7)	6	7	(4)	8	11	(5)
bleeding episodes	0	1	0	0	0	0	0	1	0	0	0	0	0		(0)	2	2	(1)	0		(0)
constipation	20	31	30	0	1	0	0	0	0	0	0	1	20	20	(13)	29	32	(19)	27	31	(18)
dyspepsia	2	2	3	0	0	0	0	0	1	0	0	0	2	2	(1)	2	2	(1)	4	4	(3)
irritation & inflammation	5	4	2	2	2	3	2	0	1	0	1	0	8	9	(5)	7	7	(5)	5	6	(3)
nausea & vomiting	34	30	30	5	11	4	1	2	3	2	3	4	26	42	(17)	30	46	(20)	24	41	(16)
other	9	6	14	1	1	3	0	0	0	1	0	0	10	11	(7)	7	7	(5)	14	17	(9)
Hematic/lymphatic system																					
anemia	85	85	86	2	1	0	0	1	1	1	0	1	79	88	(53)	75	87	(50)	74	88	(50)
other	14	20	14	1	0	0	0	0	0	0	0	0	12	15	(8)	17	20	(11)	12	14	(8)
Immune response																					
allergic reactions, surgical site	12	11	7	0	4	5	1	4	2	1	1	2	12	14	(8)	16	20	(11)	11	16	(7)
allergic reactions, systemic	13	13	24	3	2	1	0	0	0	0	1	0	14	16	(9)	12	16	(8)	16	25	(11)
autoimmune disorders	1	0	0	0	0	0	0	1	0	0	0	0	1	1	(1)	1	1	(1)	0	0	(0)
Infection																					
surgical site, superficial	24	20	17	4	3	3	1	5	1	2	2	1	25	31	(17)	22	30	(15)	20	22	(13)
surgical site, deep	15	10	14	2	3	0	1	2	1	0	1	1	16	18	(11)	13	16	(9)	14	16	(9)
general	8	9	18	4	0	1	2	0	1	3	1	1	13	17	(9)	8	10	(5)	15	21	(10)
Inflammation systemic	64	74	78	6	5	5	2	0	4	0	0	0	47	72	(31)	53	79	(35)	56	87	(38)

Adverse events reported for patients enrolled in clinical trial supporting approval

(%) of Patients
Total Adverse Events

	1st quarter postop			2nd quarter postop			3rd quarter postop			4th quarter postop			Control (n=150)			low dose (n=151)			Inves. (n=149)		
	Control	low dose	Inves.	Control	low dose	Inves.	Control	low dose	Inves.	Control	low dose	Inves.	#	Total Adverse Events	(%)	#	Total Adverse Events	(%)	#	Total Adverse Events	(%)
surgical site	72	79	67	30	24	16	6	8	10	8	3	5	78	116	(52)	78	114	(52)	71	98	(48)
Kidney	0	1	4	0	0	0	0	0	0	0	0	0	0	0	(0)	1	1	(1)	2	4	(1)
Liver, other	0	0	3	0	0	0	0	0	0	0	0	0	0	0	(0)	0	0	(0)	1	3	(1)
Metabolic & nutritional	12	13	10	1	2	0	0	1	0	1	0	0	11	14	(7)	14	16	(9)	7	10	(5)
Neuromuscularskeletal																					
altered mental status	63	56	70	1	1	4	1	0	1	1	1	3	47	66	(31)	40	58	(26)	41	78	(28)
altered sensory status, general	10	8	11	0	1	3	1	0	1	0	1	0	10	11	(7)	5	10	(3)	10	15	(7)
altered sensory status, surgical site	16	22	27	2	4	6	2	1	0	3	1	1	18	23	(12)	21	28	(14)	28	34	(19)
altered motor status, general	4	7	4	1	1	0	0	0	0	0	0	0	5	5	(3)	8	8	(5)	4	4	(3)
altered motor status, surgical site	13	11	11	2	1	1	0	0	0	1	0	0	10	16	(7)	10	12	(7)	11	12	(7)
other, surgical site	160	157	158	127	92	86	33	51	37	41	33	33	121	361	(81)	111	333	(74)	109	314	(73)
other, general	68	59	71	9	7	19	14	5	8	11	4	7	54	102	(36)	43	75	(28)	44	105	(30)
Respiratory system	31	45	39	0	2	1	0	1	0	0	1	0	30	31	(20)	38	49	(25)	32	40	(21)
Skin and appendages																					
surgical area	2	1	3	1	1	1	0	0	0	0	0	0	3	3	(2)	2	2	(1)	4	4	(3)
general	3	0	1	0	0	1	1	0	0	0	0	1	4	4	(3)	0	0	(0)	3	3	(2)
Urogenital																					
dysfunction	6	8	10	1	0	2	1	0	1	0	1	0	7	8	(5)	7	9	(5)	8	13	(5)
hematuria	0	2	3	0	0	1	0	0	0	0	0	0	0	0	(0)	2	2	(1)	4	4	(3)
infection	1	4	4	0	0	2	0	0	1	0	0	0	1	1	(1)	4	4	(3)	6	7	(4)

Potential Adverse Events:
The following is a list of potential adverse events which may occur with treatment of open tibial fractures requiring stabilization with an IM nail. Some of these adverse events may have been previously reported in the adverse events table. As with any surgery, surgical treatment of a fracture is not without risk. A variety of complications related to surgery or the use of INFUSE Bone Graft can occur. These may occur singly or in combination. Some of these may be severe, affecting patient outcome.

- Bone fracture.
- Bowel, bladder or gastrointestinal problems.
- Change in mental status.
- Damage to blood vessels, bleeding (which may require a blood transfusion) or cardiovascular system compromise.
- Damage to nearby tissues.
- Death.
- Development of respiratory problems.
- Disassembly, bending, breakage, loosening, and/or migration of IM nail components.
- Ectopic and/or exuberant bone formation.
- Fetal development complications.
- Foreign body (allergic) reaction.
- Incisional complications.
- Infection.
- Neurological system compromise.
- Nonunion (or pseudarthrosis), delayed union, mal-union.
- Pain or discomfort.
- Rash or allergic reaction.
- Scar formation.
- Side effects from anesthesia or the surgical approach.
- Swelling.
- Tissue or nerve damage.

Note: Additional surgery may be necessary to correct some of these potential adverse events.

IX. SUMMARY OF NONCLINICAL LABORATORY STUDIES

The safety of rhBMP-2/ACS has been evaluated in an extensive series of toxicology studies of both the rhBMP-2 growth factor alone and the rhBMP-2 growth factor in combination with the ACS carrier/scaffold.

In the following sections, the text describes the general results from a class of studies, while the accompanying tables identify specific studies and their outcome.

IX.1 Biocompatibility Studies

The safety of rhBMP-2/ACS was evaluated in a series of biocompatibility tests. Under the conditions of these studies, there was no mortality or evidence of significant systemic toxicity in the mouse, no intracutaneous toxicity or significant dermal irritation in the rabbit, no evidence of delayed dermal contact sensitization in the guinea pig, no evidence of cell lysis or toxicity in the extract and overlay cytotoxicity tests, no evidence of hemolysis, and no evidence of cellular mutagenicity. In the rabbit muscle irritation study, macroscopic evaluation of rhBMP-2/ACS revealed a hard, granular formation around the test site. The implant was graded macroscopically as a slight irritant relative to the negative control. Microscopically, the implant was graded as a nonirritant. The implant site revealed the presence of new bone formation consistent with the known pharmacologic action of rhBMP-2.

Study Type: Species	Groups/ No. Animals/ Sex	Route	Relevant Findings
Intracutaneous toxicity: rabbit: New Zealand white	1/2	IC	The test article extracts showed no evidence of causing significant irritation or toxicity.
Delayed contact sensitization: Guinea pig	2/15/F	ID skin patch	The test article extracts showed no evidence of causing sensitization.
Cytoxicity/ *in vitro*: WI-38 Human embryonic cell line	n/a	n/a	The test article extracts showed no evidence of causing cell lysis or toxicity.
Cytoxicity/ *in vitro* agarose overlay: L-929 mouse fibroblast cell line	n/a	n/a	The test article extracts showed no evidence of causing cell lysis or toxicity.
Systemic toxicity study/mouse	n/a	IV IP	The test article extracts were not considered systemically toxic to the mouse at the prescribed USP dosage.
In vitro hemolysis: rabbit whole blood	n/a	n/a	The test article extracts were not considered hemolytic.
Surgical muscle implantation study: rabbit/	n/a	IM	The test articles were considered to be trace-to-mild irritants after implantation in muscle.
Ames *in vitro* mutagenicity study	n/a	n/a	The test article extracts were not considered mutagenic.

n/a = not applicable

IX.1.2 Safety of rhBMP-2 Administered Intravenously: Acute and repeated dose, 28-Day Toxicity Studies

rhBMP-2 protein was studied in single- and multiple-dose general toxicology studies in the rat and beagle dog with up to 28 days of daily dosing. rhBMP-2 was administered intravenously (IV), at a range of doses that varied from slightly lower to substantially higher than the total doses (weight based) of rhBMP-2 that have been used in human clinical trials. There were no treatment-related toxicities observed in these studies. For example, although rhBMP-2 has potent bone-inducing activity at the local site of administration, systemic administration of suprapharmacologic doses of rhBMP-2 did not result in disseminated bone formation at any remote site, in any study.

Study Type: Species/ Strain	Groups/ No. Animals/ Sex	rhBMP-2 (mg/kg)	Relevant Findings
Acute toxicity: Rat/ Sprague-Dawley with sacrifices on Days 2, 7 and 15	5/ 5/sex sacrifice	saline vehicle 0.053 0.160 0.533	No toxicity observed. No-toxic-effect dose was 0.533 mg/kg IV.
Acute toxicity: Rat/ Sprague-Dawley with sacrifices on Days 2 and 15	4/ 5/sex sacrifice	vehicle 0.53 1.6 5.3	No treatment-related findings in animals sacrificed at Day 2. Slight-to-mild dose-related chondrogenesis at injection sites. No-toxic-effect dose was 5.3 mg/kg IV.
Acute toxicity: Dog/ Beagle Sacrifice on Day 15	4/ 1/sex	vehicle 0.53 1.6 5.3	No toxicity observed. No-toxic-effect dose was 5.3 mg/kg IV.
28-Day toxicity: Rat/ Sprague-Dawley	5/ 10/(5)[a]/sex	saline vehicle 0.016 0.05 0.16	Ten deaths unrelated to treatment (vehicle, 0.016, 0.05, 0.16) Dose-related soft tissue thickening and cartilage formation in subcutaneous tissue at injection sites. Following 28-day recovery period, the soft tissue thickening regressed and matured to bone. No-toxic-effect dose was 0.16 mg/kg/day IV.
28-Day toxicity: Dog/ Beagle	5 3/(2)[a] /sex	saline vehicle 0.016 0.05 0.16	Dose-related perivascular fibroplasia at injection site in all rhBMP-2-treated animals with bone formation in mid- to high-dose groups. No-toxic-effect dose was 0.16 mg/kg/day IV.

[a] () numbers of additional recovery sub-group animals in control and high-dose groups

IX.1.3 Chronic Toxicity

The long-term safety of implanted rhBMP-2/ACS was evaluated in two studies, a 6-month mandibular/maxillofacial inlay study in beagle dogs and a 1-year femoral onlay study in Sprague-Dawley rats. These studies were designed to assess the potential long-term systemic and local effects of rhBMP-2/ACS in two species at two skeletal sites. Implants of rhBMP-2/ACS had no systemic effects and local effects were associated with the osteoinductive activity of rhBMP-2. Transient, low-titer immune responses were observed in the dog study.

Study Type: Species/ Strain	Groups/ No. Animals/ Sex	rhBMP-2 (mg/kg)	Relevant Findings
6-month Mandibular/ Maxillofacial Implant (at inlay defect site): Dog/Beagle	5 2/sex sacrificed at 3 and 6 months post-implantation	sham surgery, vehicle/ACS, 0.078 mg/kg (0.4 mg/mL)/ACS, 0.312 mg/kg (1.6 mg/mL)/ACS, 0.781 mg/kg, (4.0 mg/mL)/ACS	No effects of treatment on clinical signs, hematology, or clinical chemistry. Dose-related post-surgical swelling. As swelling subsided (3-4 weeks), firm masses near the zygomatic and mandibular implant sites were detected in most rhBMP-2-treated animals. Histologically, the rhBMP-2-treated implant sites were composed of abundant fibrocellular tissue and/or new bone formation within and around the defect site. There were fluid filled tissue cysts and occasionally strands of residual ACS material at implant sites with apparent regression between 3 and 6 months. Implant site changes were expected exaggerated pharmacologic responses to rhBMP-2/ACS and were not toxicologically significant. No-toxic-effect dose was 0.781 mg/kg (4.0 mg/mL concentration rhBMP-2). Transient low titer antibody responses were observed in 15/24 (62.5%) of the treated animals.
1-year Femoral onlay Implant Toxicology: Rat/Sprague-Dawley	5 10/sex sacrificed at 1, 6, and 12 months post-implantation	vehicle/ACS, 0.04 mg/kg (0.1 mg/mL)/ ACS, 0.3 mg/kg (0.75 mg/mL)/ ACS, 1.6 mg/kg (4.0 mg/mL)/ ACS	Slight increased incidence and severity of surgical site swelling at 1.6 mg/kg. Dose-related pharmacologic effect of increased incidence and/or severity of bone formation at implant site in all rhBMP-2/ACS treatment groups. No toxicity at any dose.

IX.1.4 Safety of rhBMP-2 Administered Intravenously: Fertility, General Reproductive Performance and Teratology

Because BMP-2 participates in embryological development, rhBMP-2 was evaluated for any effect on reproduction or fetal development. Studies evaluated rhBMP-2 at total doses (weight based) that ranged from slightly lower to substantially higher than rhBMP-2 doses that are anticipated in clinical use (up to approximately 1 mg rhBMP-2/kg, total delivered dose). The effects of rhBMP-2 on the reproduction and fertility of male and female Sprague-Dawley rats was studied. Maternal and paternal mating performance and reproductive parameters were not affected by treatment. Range-finding studies followed by developmental toxicity studies were conducted in both Sprague-Dawley rats and New Zealand white rabbits. There was no evidence of maternal toxicity, embryolethality, fetotoxicity, or teratogenicity.

Study Type: Species/ Strain	Groups/ No. Animals/ Sex	rhBMP-2 (mg/kg)	Relevant Findings
Fertility: rat/Sprague-Dawley	5/ 40/F 40/M	saline vehicle 0.016 0.05 0.16	Maternal and paternal mating performance and reproductive parameters were not affected by treatment. No-toxic-effect dose was 0.16 mg/kg/day IV.
Range-finding Teratology: rabbit/ New Zealand white rabbit	7/ 5/F	saline vehicle 0.016 0.05 0.16 0.5 1.6 Days 6 to 18 gestation	No maternal toxicity, embryolethality, or gross fetal abnormalities. No-toxic-effect level was 1.6 mg/kg/day IV.
Teratology: rabbit/New Zealand white	5/ 20/F	saline vehicle 0.016 0.5 1.6 Days 6 to 18 gestation	No maternal toxicity, embryolethality, or gross fetal abnormalities. No-toxic-effect level was 1.6 mg/kg/day IV. Definitive teratology study in rats. The incidences of malformations were not significantly different between control and treated groups.
Range-finding Teratology: Rat/ Sprague-Dawley	7/ 6/F	saline vehicle 0.016 0.05 0.16 0.5 1.6 Days 6 to 17 gestation	No maternal toxicity, embryolethality, or gross fetal abnormalities. No-toxic-effect level was 1.6 mg/kg/day IV.
Teratology: Rat/ Sprague-Dawley	5/ 25/F	saline vehicle 0.16 0.5 1.6 Days 6 to 17 gestation	No maternal toxicity, embryolethality or fetal abnormalities. No-toxic-effect dose was 1.6 mg/kg/day IV. The definitive teratology study in rats was repeated. In the initial study there was a nonsignificant difference in skeletal formation between the rhBMP-2 groups and the saline and control groups. Examination of skeletal variance revealed a significant reduction in sternebral variance in all treated groups.

Study Type: Species/ Strain	Groups/ No. Animals/ Sex	rhBMP-2 (mg/kg)	Relevant Findings
Repeat teratology: rat/Sprague-Dawley	2/ 25/F	vehicle 1.6 Days 6 to 17 gestation	It was hypothesized that the difference in skeletal formation in the preceding study was a result of the time of cesarean section rather than a treatment effect. This repeat study had a random order of selection for time of cesarean section. No maternal toxicity, embryolethality, fetotoxicity or teratogenicity and no difference in skeletal formation between the control and rhBMP-2 groups. No-toxic-effect level was 1.6 mg/kg/day IV.

IX.1.5 Carcinogenicity/Genotoxicity

In addition to the Ames Mutagenicity assay, the sponsor investigated the potential for rhBMP-2 to stimulate the proliferation of primary tumor cell isolates and tumor cell lines. rhBMP-2 was examined for growth potentiating activity *in vitro* on human tumor cell lines and primary tumor cell isolates at concentrations of 10 to 1000 ng/mL. No growth potentiating activity was observed. rhBMP-2 exhibited growth inhibition of several carcinoma-derived tumors. The studies did not include analyses to determine if the cells expressed BMP type I and II receptors. Overall, studies investigating the potential effects of rhBMP-2 on tumor cell growth showed minimal evidence of growth potentiation including studies of osteosarcoma cell lines.

Study Type: Species/ Strain	Groups/ No. Animals/ Sex	rhBMP-2 (mg/kg)/Route	Relevant Findings
Growth potential on primary tumor isolates *in vitro* (Soda et al., *Anti-Cancer Drugs*, 1998)	n/a	10, 100, and 1000 ng/mL concentration *in vitro*	No tumor cell growth stimulation. Significant inhibition of colony forming units in 16 of 65 specimens at 1000 ng/kg
Inhibition of tumor growth *in vitro* with human tumor cell lines	n/a	10, 100, and 1000 ng/mL concentration *in vitro*	No effect on osteosarcoma cell line growth. Inhibitory effects on several soft tissue carcinoma cell lines.

IX.2 Immunology

Formation of antibodies to rhBMP-2 and Type I collagen in canines, rhesus monkeys and rats was monitored using Enzyme Linked Immusorbent Assays (ELISAs). In general, rhBMP-2 or bovine Type I collagen was coated onto microtiter plates. Controls or serum samples were diluted in assay buffer and incubated on the plates. Enzyme-conjugated reagents were used to detect bound antibodies. Enzyme substrates were then incubated on the plates and optical densities of the solutions were measured in order to quantitate the presence of anti-rhMBP-2 or Type I collagen antibodies. Immune responses to rhBMP-2 were observed in nonhuman primates and in dogs. Please refer to the 6 month beagle dog study in section IX.2.1.3, Chronic Toxicity, and the radius, critical size defect repair/nonhuman primate study in section IX.2.5, Animal Studies.

IX.3 Pharmacokinetics

IX.3.1 rhBMP-2 Protein Administered Intravenously

Although rhBMP-2 is intended to be delivered with the ACS as two part device component, pharmacokinetic results obtained from IV dosing provide a means to evaluate the extent and duration of systemic exposure of rhBMP-2. Studies were conducted to characterize the pharmacokinetics of rhBMP-2 in the blood of rats and monkeys. The uptake of rhBMP-2 by highly perfused tissues and organs is rapid, but the residence of the protein is short. Catabolism of the protein is extensive and renal excretion of trichloroacetic acid-soluble radioactivity is rapid. rhBMP-2 is rapidly eliminated in rat and nonhuman primates ($t_{½}$ = 16 minutes in the rat and $t_{½}$ = 6.7 minutes in nonhuman primates) from the systemic circulation following intravenous administration. A study conducted in juvenile and adult Sprague-Dawley rats revealed that juvenile rats, like adult rats, cleared rhBMP-2 rapidly. Results also showed a lower maximal concentration, higher clearance and a larger initial volume of distribution for rhBMP-2 in juvenile rats as compared to adult rats. As a result of these pharmacokinetic characteristics, systemic presence of rhBMP-2 in the circulation is minimal after IV dosing.

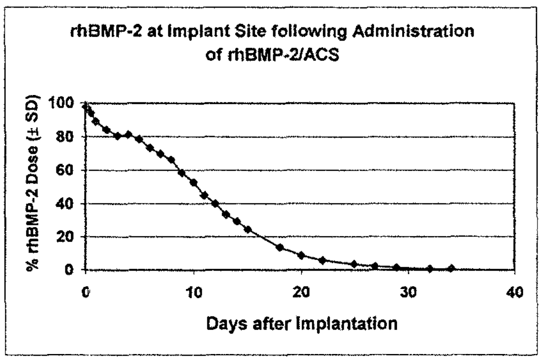
Study Type/ Species/ Strain	Groups/ No. Animals (per group)	rhBMP-2 Dose	Relevant Findings
PK single dose: rat/Sprague-Dawley	4/3	0.43 4.3 43 860 µg/kg	Clearance of ^{125}I-rhBMP-2 was rapid and biexponential: $T_{1/2\alpha}{}^{a}$ = 0.8 min, and $T_{1/2\beta}{}^{b}$ =15.3 min. Most of the administered dose (92%) was recovered by 24 hours in the urine as TCA-soluble counts per minute. ^{a} $T_{1/2\alpha}$ = half-life of initial phase ^{b} $T_{1/2\beta}$ = half-life of terminal phase
PK single dose: nonhuman primate/ cynomolgus monkey	2/3	4.9 µg/kg	Clearance of ^{125}I-rhBMP-2 was rapid and biexponential: $T_{1/2\alpha}{}^{a}$ = 1.0 min, and $T_{1/2\beta}{}^{b}$ =7.0 min.
Biodistribution: rat/Sprague-Dawley	8/3	4.3 µg/kg	Rapid localization to liver with metabolism and excretion into urine. Biphasic disposition was observed with initial and terminal half-life of 0.8 and 31 minutes, respectively.

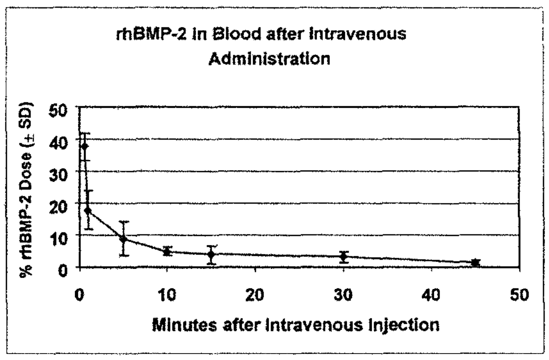
Study Type/ Species/ Strain	Groups/ No. Animals (per group)	rhBMP-2 Dose	Relevant Findings
Biodistribution: rat/Sprague-Dawley	8/3	7.1 μg/kg	^{125}I-rhBMP-2 rapidly distributed to the highly perfused tissues; 1 minute after dosing, 82.4% of the dose was recovered in the liver, lung, kidney, and spleen. The liver was the predominant site of ^{125}I-rhBMP-2 localization throughout the study.
PK single dose: juvenile and adult rats/Sprague-Dawley	2/12-24	3.0 mg/kg	Clearance of ^{131}I-rhBMP-2 was rapid and biexponential in both juvenile and adult rats as assessed by serum acid precipitable radioactivity and by ELISA.

IX.3.2 Local Retention of rhBMP-2 Administered With ACS

The local residence time of rhBMP-2 when applied to the ACS was assessed following subcutaneous (SC) implantation in rats and implantation at orthotopic sites in rats and rabbits. The results from all three models were similar. In the rat femoral onlay model, ^{125}I-rhBMP-2 was slowly released from the implant site with a mean residence time of approximately 8 days (refer to Figure below). The peak amount of radiolabeled rhBMP-2 detected in the blood was small, 0.1% of the implanted dose, and consistent with the rapid systemic clearance described above.

Retention of rhBMP-2 Following Implantation of rhBMP-2/ACS (left) and Pharmacokinetic Evaluation Following Intravenous Administration of rhBMP-2 (right) in the Rat





IX.3.3 Interactions Between rhBMP-2 and the Absorbable Collagen Sponge

The importance of retaining rhBMP-2 at the implant site for optimal bone formation was highlighted in one study in which the heparin-binding domain of rhBMP-2 was removed using plasmin. Following implantation with ACS, this modified protein was found to leave the implant site much more rapidly than the native protein; for example, only 18% of the plasmin-cleaved material was present at 3 hours, contrasting with 56% for the native protein. Though the plasmin-cleaved protein was highly active in a cell culture assay, bone formation *in vivo* was substantially reduced, indicating that retention of bioactive material at the implant site is critical for the desired osteogenic activity.

As the local retention of rhBMP-2 is important for localized bone formation, several studies evaluated the effect of potential variables on retention following SC implantation in the rat or orthotopic implantation in the rabbit. The relative retention of rhBMP-2 was unaffected by the concentration of rhBMP-2 administered (between 0.8 and 2.0 mg/mL) or the formulation buffer. The amount of rhBMP-2 incorporated into ACS (a measure of the binding of rhBMP-2 to the sponge prior to implantation) had a minimal effect on rhBMP-2 retention *in vivo*, and no effect on the rate of release of rhBMP-2 into serum *in vitro*. These results suggest that the release of rhBMP-2 *in vivo* is independent of the binding of rhBMP-2 to the sponge *in vitro*, and may be diffusion-controlled.

IX.4 Effect of Nicotine and Glucocorticosteroids on Implantation with rhBMP-2/ACS

In animal studies, the sponsor has shown that rhBMP-2/ACS induces bone and fracture repair in the presence of several agents which compromise bone metabolism, for example, nicotine and corticosteroids.

Study Type/ Species	Groups/ No. Animals	rhBMP-2 (mg/mL)	Relevant Findings
Subcutaneous implant/rat	8[a]/4-5	0 0.01 0.1 0.4	Systemic nicotine treatment did not inhibit the ability of rhBMP-2/ACS to induce bone formation.
Ulnar osteotomy repair/rabbit	3[b]/12-13	0.2	Nicotine did not affect the rate of fracture repair in this model.
Subcutaneous implant/rat	8[c]/6	0 0.01 0.1 0.4	Prednisolone treatment dramatically inhibited bone growth and body mass gain. Prednisolone treatment also inhibited ectopic bone formation; however, the high dose of rhBMP-2/ACS overcame this inhibition.
Ulnar osteotomy repair/rabbit	4[d]/12	0 0.2	Prednisolone treatment inhibited fracture healing. Treatment with rhBMP-2/ACS overcame this inhibition and enhanced healing in both the control and prednisolone treated animals.

[a] four nicotine-treated; four control
[b] nicotine-treated; nicotine pre-treated, or control
[c] four prednisolone-treated; four control
[d] prednisolone-treated or control; two sacrifice timepoints

IX.5 Animal Studies

Pharmacology studies have demonstrated that rhBMP-2/ACS can induce bone and repair large, segmental critical-sized defects in rat femora, rabbit radii and ulnae, dog radii, and nonhuman primate radii. The induced bone biologically and structurally integrates with the pre-existing bone, and remodels physiologically, *i.e.*, consistent with the biomechanical forces placed on it. In addition, the rhBMP-2/ACS-induced bone can repair itself following fracture, in a manner indistinguishable from native bone. Separate studies demonstrated that rhBMP-2/ACS can accelerate healing in rabbit and goat long bone fracture models. Thus, radiographic, biomechanical, and histologic evaluation of the induced bone indicates that it is appropriate for the anatomic site where it forms, and functions biologically and biomechanically as native bone.

Histologic analyses from many pharmacology studies have characterized the cellular events involved in the bone induction process initiated by rhBMP-2/ACS. Mesenchymal cells from the surrounding tissues first infiltrate the periphery of the rhBMP-2/ACS implant. As the ACS is degraded, these cells appear to differentiate and begin to form trabecular bone and/or cartilage. Vascular invasion is evident at the same time. The bone formation process temporally extends from the periphery of the rhBMP-2/ACS implant towards the center, until the entire rhBMP-2/ACS implant is replaced by trabecular bone. Remodeling of the trabecular bone then occurs depending on the physiologic form of the site and function applied to the bone. This ability of rhBMP-2/ACS to support bone remodeling may also be responsible in part for the biologic and biomechanical integration of the new bone induced by rhBMP-2/ACS with that of the surrounding bone.

Study Type/ Species	**Groups/No. Animals (per group)**	**rhBMP-2 (mg/mL)**	**Relevant Findings**
Subcutaneous implant/rat	5/6	0 0.17 0.33 0.66 1.7	Substantial bone induction was observed in all implants containing rhBMP-2.
Femur critical sized defect repair/rat	6/7	0.013 0.025 0.05 0.1 0.2 0.4	Dose-response-related healing of critical-sized bone defects measured by radiologic and histologic criteria. Therapeutic dose (concentration) range of 0.025 – 0.05 mg/mL rhBMP-2/ACS delineated for this model.
Radius critical-sized defect repair/dog	4/3	0 0.05 0.2 0.8	All rhBMP-2/ACS-treated animals healed at 12 weeks by all criteria. Dose-dependent generation of excess bone and voids; voids observed especially in bone formed outside of implant area. Biomechanical values of all rhBMP-2 dose groups are equal or superior to autologous bone-grafted controls.
Radius critical-size defect repair/dog	3/5	0 0.05 0.2	Functional loading of rhBMP-2/ACS-treated radii at 16 week demonstrated. Remodeling and consolidation of induced bone observed during subsequent 8-32 week. Remodeling over time of void spaces observed. Stress fractures in rhBMP-2/ACS and autogenous bone grafted limbs occurred at similar frequencies and healed at similar rates.

Study Type/ Species	Groups/No. Animals (per group)	rhBMP-2 (mg/mL)	Relevant Findings
Radius critical-size defect repair/nonhuman primate	9/2-6	0 0.05 0.2 0.4 0.8 1.3 1.5 1.9 3.1	Variable bone induction observed in range of 0.4 to 1.5 mg/ml rhBMP-2/ACS. No dose-response defined. No radiolucent voids generated at any dose. Dose-related generation of antibodies to rhBMP-2 were observed in 35% (7/20) treated. Antibodies to Type I collage were observed in 7% (1/14); no correlation with efficacy.
Ulnar critical-size defect repair/nonhuman primate	6[a]/1	0 0.75	Bone induction within defect by 4 weeks. Accelerated removal of ACS in presence of rhBMP-2. Compression of ACS limited amount of bone induction observed.
Femoral head core defect/sheep	3[a]/2	0 0.4 1.5	Superior retention of rhBMP-2, bone induction within defect and bone formation surrounding defect when implanted with ACS as compared to injection in buffer. rhBMP-2/ACS induced early remodeling in trabecular bone surrounding implantation site.
Femoral head core defect/sheep	2[b]/2	0 0.8 1.5	Superior bone induction observed in response to rhBMP-2/ACS compared to ACS alone or surgical control. Early bone resorption surrounding defect observed followed by subsequent bone formation. A positive correlation was found between retention of rhBMP-2 at the implant site and bone formation.
Ulnar osteotomy repair/rabbit Pilot I[b] Pilot II[b] Main	many/5-15 3[a]/10 2[c]/15 6/15	0 0.05 0.1 0.2 0.4 0.8	Increase in torsional loading parameters with rhBMP-2/ACS placed as an onlay. rhBMP-2/ACS-treated limbs healed at 3-4 weeks, as compared to 6 weeks in controls.
Ulnar osteotomy repair/rabbit	2/6[d]	0 0.2	Torsional biomechanics in the rhBMP-2/ACS group 80-100% greater than surgical control and ACS groups at 3 and 4 weeks. No difference between surgical and ACS onlay groups at any time point. Approximately 25% decrease in time to bony union with rhBMP-2/ACS.
Tibial fracture repair/goat	2/1-2	0.2 0.8	No obvious differences in treated and control fractures. Conclusions difficult to reach due to small sample size and lack of carrier control groups.

Study Type/ Species	Groups/No. Animals (per group)	rhBMP-2 (mg/mL)	Relevant Findings
Tibial fracture repair/goat	2/4	0 0.4	rhBMP-2/ACS used as an onlay resulted in increased callus size and maturity, as assessed radiographically and histologically. Fractures wrapped with rhBMP-2/ACS had increased torsional toughness.
Femoral allograft incorporation/dog	3/7	0 0.4	Augmentation of the allograft-host bone junctions with rhBMP-2/ACS resulted in a stronger and more complete union than with autogenous bone graft or ACS alone.
Radius defect repair/rabbit	4/8	0 0.1 0.4 1.3	No deleterious effects on radiocarpal joint observed when rhBMP-2/ACS placed in a distal radius defect connected to joint cavity.
Mid-diaphyseal Ulnar Osteotomy repair/rabbit	4[e]/6	0.4	Histologically there was no treatment effect on morphology of the growth plates.
Ulnar osteotomy repair/rabbit	7[f]/3-9	0.2	As assessed by radiography and histology, defects in bone induced by rhBMP-2/ACS heal by a process similar to that seen in normal bone.

[a] sacrifice at three timepoints
[b] contralateral control = ACS or not treatment
[c] sacrifice at two timepoints
[d] at each of four timepoints
[e] two or three month old rabbits; rhBMP-2/ACS or surgical control
[f] seven timepoints

IX.6 Preclinical Effectiveness Evaluations Conclusions

Many of the animal pharmacology studies have included dose-ranging, and from these results, a broad therapeutic concentration range, measured as quantity of rhBMP-2 per unit volume of ACS, has been determined. This therapeutic range is bordered on one side by inadequate bone formation and on the other by excessive bone formation combined with an increased incidence and size of fluid-filled void spaces within the induced bone (both of which remodeled appropriately over time). The therapeutic rhBMP-2 concentration range shifts with the animal species tested in apparent accord with the bone formation rate of that animal. Thus, higher concentrations are required in dogs than in rats, and even higher concentrations in nonhuman primates. The upper end of the therapeutic concentration range in nonhuman primates has not been defined by these studies. The nonhuman primate therapeutic range, 0.4 – 1.5 mg/mL rhBMP-2, is the same that has been tested in humans. Both the concentration of rhBMP-2 and the length of time that rhBMP-2 is present at the implant site are positively correlated with the rate of bone formation, the amount of bone formed, and the density of the resulting bone.

In summary, the safety (toxicology and pharmacokinetics) and bone-forming capacity of rhBMP-2/ACS have been extensively investigated and are well understood. The nonclinical safety of systemically delivered rhBMP-2 and locally delivered rhBMP-2/ACS has been extensively studied and no toxicities have been identified in these studies. The disposition of rhBMP-2 and rhBMP-2/ACS is characterized by slow release of rhBMP-2 from the implantation site and rapid systemic clearance. This profile results in minimal systemic exposure to rhBMP-2. Application of rhBMP-2/ACS results in the induction of normal bone locally at the site of implantation. This process includes the migration of mesenchymal cells into the site, their proliferation and apparent differentiation into bone-forming cells. The bone induced by rhBMP-2/ACS remodels and assumes the structure appropriate to its location and function, as would be expected from host bone.

X. SUMMARY OF CLINICAL STUDIES

X.1 Study Background

Safety and effectiveness of INFUSE Bone Graft were evaluated as part of a prospective, randomized, controlled, multinational (11 countries), multi-center (49 sites) study. Subjects were randomized into one of three groups – control or one of two investigational groups (0.75 or 1.50mg/ml rhBMP-2). All subjects received wound management and fracture stabilization with an IM nail, while the investigational subjects also received INFUSE Bone Graft at the fracture site. No restrictions were placed on the type of IM nail used or whether it was reamed or non-reamed. The use of bone wax, Gelfoam, or other collagen hemostatic agents, corticosteroids, bone growth stimulators (electrical, ultrasound, or magnetic) was specifically prohibited. Only the subjects and an independent radiology panel were blinded with respect to treatment. The investigators were aware of the treatment assignment.

X.2 Inclusion criteria

The indication studied was acute, open tibial shaft fractures (Gustilo grade I, II, IIIA or IIB) with the major component of the fracture being diaphyseal. Patients with isolated tibia fractures and those with multiple injuries were included. The additional inclusion criteria were:

- age ≥18 years
- females of non-childbearing potential or who have a negative pregnancy test with 72 hours; or males
- definitive fracture stabilization with IM nail (may have temporary external fixation that is later converted to IM nail)
- definitive wound closure (DWC) within 14 days of initial injury
- DWC was the final planned surgical exposure
- in bilateral open tibia fractures, the random treatment assignment was for the right tibia
- all fracture lines were accessible through the same wound at DWC to allow treatment with one device

X.3 Exclusion criteria

Subjects were excluded if they had any of the following:

- Glasgow Coma Scale <15 at the time of informed consent
- purulent drainage from the fracture, or evidence of active osteomyelitis
- anticipated treatment plan includes procedures to promote fracture healing, *e.g.*, bone grafting, IM nail dynamization, ultrasound, magnetic field, or electrical stimulation
- initial injury occurred more than one day before initial wound debridement and fracture immobilization
- fracture had been treated with additional fixation, *e.g.*, plates, wires, or screws
- compartment syndrome
- pathological fractures; history of Paget's disease or other osteodystrophy; or history of heterotopic ossification
- history of malignancy, radiotherapy, or chemotherapy for any malignancy within the last 5 years (except basal cell carcinoma of skin)
- autoimmune disease, *e.g.*, lupus
- history of prior exposure to silicone or injectable collagen implants
- hypersensitivity to protein pharmaceuticals, *e.g.*, monoclonal antibodies, gamma globulins, or collagen
- treatment with any investigational therapy within 28 days of implantation surgery
- treatment for 7 days or more with a drug that interferes with bone metabolism, *e.g.*, prednisone (cumulative dose >150mg within 6 months), calcitonin (within 6 months), bisphosphonates (for 30 days or more within 12 months), therapeutic doses of fluoride (for 30 days within 12 months), therapeutic doses of vitamin D (for 30 days within 6 months)
- if female, subject was nursing

X.4 Post-operative care

No recommendations were made with respect to a common post-operative rehabilitation regimen.

X.5 Clinical and radiographic effectiveness parameters

Subjects were followed for 12 months after definitive wound closure (DWC). Evaluations were performed postoperatively at 6, 10, 14, 20, 26, 39 and 50 weeks, however, the protocol did not include definitions of the evaluation time windows. Adverse events, device-related or not, were evaluated over the course of the clinical trial. At each evaluation timepoint, the primary and secondary clinical and radiographic outcome parameters were evaluated. Success was determined from data collected during the initial 12 months of follow-up. Antibodies to rhBMP-2 and bovine Type I collagen were assessed preoperatively and at timepoints post-operatively. Antibodies to human Type I collagen were assessed if the antibody response to bovine Type I collagen was positive.

Clinical and radiographic fracture assessments were performed at each postoperative visit; however the protocol did not provide the specific objective criteria that were used to determine fracture healing. The "Assessment of Fractured Limb" case report form provided for the documentation of the following parameters, but did not indicate how these were to be used to determine fracture status or how many needed to be present for in order for a complete evaluation to have occurred :

- wound (healed, not healed, not evaluated)
- pain (absent, present, not evaluated)
- swelling (absent, present, not evaluated)
- tenderness (absent, present, not evaluated)
- neurovascular status (intact, impaired, not evaluated)
- infection (absent, present, not evaluated)
- weight-bearing status (non-, touch down, partial, full, not evaluated).

Radiographic assessments (AP and lateral radiographs) were performed at each post-op visit. Oblique radiographs were to be used if the standard views did not adequately visualize the fracture. The radiographs were evaluated by the investigator and the radiology panel.

Investigators were to evaluate radiographic healing by assigning a score of "united", "not united", "uncertain union" or "uninterpretable". No definitions for these terms were provided in the protocol.

The protocol for the independent radiology panel stated that "...fracture union was determined if there was cortical bridging and/or disappearance of the fracture lines were visible on 3 of the 4 bone aspects (anterior, posterior, medial, and lateral)..." These definitions were not available to the investigators. The first visit at which these criteria were met was considered the time of union radiographically. The independent radiographic evaluation protocol called for the review of each radiograph by all three members of the panel. An agreement of 2 of the 3 reviewers was necessary for a determination of fracture union. The independent radiographic evaluation was performed on all available radiographs. Because the investigators determined whether a subject required a secondary intervention to promote fracture healing at any time during the study, a complete set of radiographs on all subjects for the entire study duration might not be available to the radiology panel for all enrolled subjects.

Adverse events were assessed for relatedness to the device and severity was based on the WHO recommendations.

Investigators were provided with the following definitions:

> "...*Nonunion* – considered to be established when a minimum of 9 months have elapsed since injury and the fracture site showed no visibly progressive signs of healing for a minimum of 3 months (no change of fracture callus).
>
> *Delayed union* – insufficient fracture healing determined by radiographic and clinical assessment. A specific time point at which delayed union was defined was not provided.

Secondary intervention for delayed union – any intervention, surgical or non-surgical, that was performed to induce or accelerate fracture union after DWC. Examples included use of autograft, allograft or bone graft substitutes; IM nail dynamization; exchange nailing; or noninvasive modalities, *e.g.*, ultrasound, magnetic field, or electrical stimulation...." The decision to perform a secondary intervention for delayed union was dependent on the definition of delayed union above.

Investigators determined fracture union based on clinical judgement. The protocol did not provide the specific objective criteria that were used to determine fracture healing or deciding whether to recommend secondary interventions to promote fracture healing.

X.6 Patient demographics and accountability

The sample size estimation called for 150 subjects per treatment group. A total of 149 investigational and 150 control patients were enrolled in the study and received treatment. Only the results from the control patients and investigational patients receiving the 1.5mg/ml dose device are described below as part of the effectiveness evaluation.

The demographics of the patient population were similar across all study groups except for the parameter of age, specifically the mean and range. The subjects in the investigational group were younger (mean = 33.4 years, range 18-77 years) compared to the control group (mean = 36.8 years, range = 17-87 years). Patients in the control group had a slightly larger percentage of nails that were unreamed and/or less than 9mm, while the investigational group had a higher percentage of reamed nails and /or nails that were greater than 11mm. Nail type did affect the number of secondary interventions, *i.e.*, patients with unreamed nails had a higher incidence of secondary interventions compared to patients receiving reamed nails.

X.2.7 Clinical and radiographic effectiveness evaluation

The primary efficacy endpoint was defined as the proportion of subjects who required a secondary surgical intervention to promote fracture healing within 12 months of DWC. The secondary efficacy endpoints included the following:

- the proportion of subjects healed at 6 months without a secondary intervention as determined by the investigator's clinical and radiographic assessment;
- the independent radiology panel's assessment of time to fracture union; and
- the pharmacoeconomic impact of the treatment.

The rate of secondary interventions was significantly lower in the INFUSE Bone Graft group (p=0.001) as described in the table below. Interventions were categorized in one of three ways – recommended by the investigator and performed, recommended by the investigator and not performed, or not recommended by the investigator but performed anyway. If any of these occurred, the patient was considered to have failed the primary endpoint and, therefore, was considered a study failure. In addition, patients who experience screw breakage resulting in self-dynamization were also considered treatment failures.

Number of patients with secondary interventions[a]		
	control (n = 150)	investigational (n = 149)
Recommended/performed	38	19
Recommended/not performed	3	5
Not recommended/performed	6	7
Self-dynamizations	19	7
Total failures/patients (%)	66/150 (44)	38/149 (26)

[a]Includes bone grafting, fibula osteotomies, exchange nailing, plate fixation, Ilizarov frame removal, external fixation placement, bone transport, IM nail dynamizations, exchange to a functional brace and electrical stimulation

X.2.8 Safety and immune response evaluation

The assessment of safety consisted of an evaluation of the reported adverse events, as well as an evaluation of antibodies to rhBMP-2, bovine Type I collagen and human Type I collagen. The complete list of adverse events is described in the Adverse Events section above. Refer to this section for a description of the rates associated with infection and abnormal clinical lab values. Adverse events of special interest are discussed below.

fracture healing

The rates of hardware failure in the investigational and control groups were 18/149 (14%) and 32/150 (24%), respectively. Delayed union was the most frequent serious adverse event reported at one year; occurring in 39 (26%) control and 26 (17%) investigational patients.

The rate of nonunion was lower in the investigational group as compared to control. A total of 80/150 (53.3%) control and 56/149 (37.6%) investigational patients did not require a secondary intervention and were not radiographically healed at 12 months as determined by the independent radiology panel. For the control patients who required a secondary intervention, 18/66 (27%) reported nonunions at 12 months compared to 19/38 (50%) investigational patients. Investigational patients who required a secondary intervention were considered healed *later* than control patients.

abnormal bone formation

Heterotopic ossification (HO) was not a significant concern and no ectopic ossification was reported. Because only the involved tibia was evaluated radiographically, it is not clear if abnormal bone formation occurred in other anatomical locations. Although twice as many investigational patients reported hypertrophic callus formation compared controls (8 vs. 4), no action was required to treat any of these events. A total of 12 patients experienced at least one event classified as hypertrophic callus, with the investigational group having the highest number (8) and percentage (6%) of patients with HO. No interventions were required to treat any of the HO-related events.

infections

The combined rate of deep and superficial infections of the injured limb was lower in patients with Gustilo IIIA and B fractures of the investigational group as compared to the control group [16/66 (24%) and 26/61 (43%), respectively].

immune response

The presence of antibodies was assessed using ELISA. If there was a positive response to bovine Type I collagen, the serum was also tested for antibodies to human Type I collagen. The screening ELISA cutpoint for positive antibody responses was set to 2 times the signal generated by pooled normal human sera in each ELISA. Subjects were considered to have an elevated immune response if the preoperative test was negative (titer < 50) and postoperative test was positive (titer ≥ 50) or if the preoperative test was positive and the postoperative test was positive with a three-fold higher titer than the preoperative test.

There were detectable rhBMP-2 antibodies in 1 control patient and 9 investigational patients after treatment. Of the 9 investigational patients with elevated post-treatment antibody titers, 2 were elevated at visit 6 (20 weeks), the last planned assessment and data from 1 patient were unavailable for visit 6. An additional sample from 1 of these patients was collected and tested. Following the positive test at visit 6, and the titers decreased to <50 (No follow-up data were available for the other patient. Anti-rhBMP-2 antibody responses were determined to be transient in 6/9 patients by 20 weeks, and in 7/9 patients after follow-up testing (samples from 2 patients were unavailable to confirm transience of the antibody response). Because of the small numbers involved, it was not possible to determine if a correlation existed between the immune response and clinical outcome.

There were 38 patients who developed antibodies to bovine Type I collagen - 9 (6%) control and 29 (20%) investigational patients. Approximately half of the patients had persistently elevated antibody titers at evaluations 20 weeks and more after DWC. Thirty categories of adverse events that may have been manifestations of an immune response were identified and all were observed to have a comparable incidence across all groups. Although there were 4 patients with an adverse event termed "allergic event", the investigators believed that there was no evidence of allergic response to the investigational treatment.

Immune response		
	control [n (%)]	investigational [n (%)]
anti-rhBMP-2 antibodies	1 (1)	9 (6)
anti-bovine Type I collagen antibodies	9 (6)	29 (20)
anti-human Type I collagen antibodies	0 (0)	0 (0)
# healed patients with antiBMP-2 antibody response (successes)	1	6
# secondary intervention patients with antiBMP-2 antibody response (failures)	0	3

The rates of authentic antibody response to rhBMP-2 were higher than that observed for another application of rhBMP-2/ACS. When rhBMP-2/ACs was placed inside of a metallic spinal fusion cage for anterior interbody fusion treatment of degenerative disc disease, the antiBMP-2 antibody response in the investigational group was 0.7%. This compares to a 6% rate in the investigational group in the trauma study. The contribution of the trauma setting to this outcome is unknown, as is the clinical significance of the antibody response.

XI. CONCLUSIONS DRAWN FROM THE STUDIES

All of the data provided in the previous sections describing the preclinical and clinical studies provide reasonable assurance of the safety and effectiveness of INFUSE Bone Graft when used by well-trained surgeons to treat acute, open tibial shaft fractures that have been stabilized with IM nail fixation after appropriate wound management in skeletally mature subjects within 14 days of the initial fracture. The clinical study demonstrated that patients receiving the recommended higher dose required fewer additional medical procedures to promote healing after implantation of the product compared to the group of patients who did not receive the product. Patients who received the product and required an additional medical procedure, however, healed at a slower rate compared to patients who did not receive the product.

XII. PANEL RECOMMENDATION

The PMA was reviewed at the Orthopedic and Rehabilitation Devices Advisory Panel meeting held on November 21, 2002. In a 6-1 vote, the Panel recommended to the FDA that the application for INFUSE Bone Graft was approvable on the following conditions:

- use of the device should be limited to open fractures that are "problematic";
- surgeon education should include information on the potential benefit, as well as the potential risks associated with the use of osteogenic proteins;
- the labeling should clearly describe that the risks associated with repeat use of the device are unknown;
- the sponsor should perform the following nonclinical post approval studies:
    - an evaluation of BMP receptor expression determination and *in vitro* proliferation evaluation in primary tumor cell isolates;
    - an evaluation of the ability of rhBMP-2 antibodies to cause reproductive problems in all stages of fetal development from implantation to birth; and
    - an evaluation of the response of mice to equal, multiple does of rhBMP-2 over a 1 year period;
- the sponsor should perform two studies related to antibody expression:
    - a natural history study in the patients already exposed to the device as described in the PMA; and
    - prospective studies in an appropriate animal model and a new clinical population;
- the sponsor should initiate a post market clinical study to assess the ability of the device to accelerate healing; and

- the sponsor should perform a statistical evaluation of the effect of tibial fixation on success for the population described in the PMA.

The conditions related to surgeon education, labeling, nonclinical post approval studies and antibody expression were not discussed directly by the Panel during this meeting. The Panel included these conditions by reference to the January 10, 2002 meeting where they had discussed a device that contained this device as a component.

XIII. CDRH DECISION

CDRH concurred with the Panel's approvable recommendation, however we did not agree with all of the conditions of approval. CDRH concurred with the Panel's recommendations regarding:

- additional studies involving determining primary tumor cell BMP receptor status and primary tumor cell *in vitro* responsiveness to rhBMP-2

- experiments to evaluate the potential for maternal antibodies to rhBMP-2 to interfere with embryonic development;

- device labeling;

- surgeon training; and

- the impact of tibial fixation on success.

The agency did not agree with the recommendation to evaluate toxic effects due to multiple doses of rhBMP-2 in mice over a one-year period for the following reasons:

- The sponsor had already conducted a one-year rat study and a 6-month dog study and found no device-related toxicities;

- The device is not intended to be implanted multiple times in prospective patients. The one-year rat study conducted by the sponsor evaluated the product under conditions analogous to that for which the product was intended to be used.

- The sponsor has conducted many animal studies to evaluate device performance. Repeat administration of rhBMP-2 in dogs and rats was evaluated to 28 days post-exposure. CDRH believes that the rodent model suggested by the Panel would not provide additional safety information regarding the product that has not already been obtained from other studies.

The agency did not agree with the recommendation to allow the device to be used for any fracture that was determined to be "problematic". The approved indication reflects the use of the device that was evaluated in the sponsor's clinical trial.

The agency did not agree with the recommendation to perform the two antibody response natural history studies or the accelerated healing clinical study described by the Panel. While the natural history studies could provide useful information with respect to antibody levels over time, it is difficult to follow patients for the required length of time and accurately collect the very time-dependent data to address this issue. As demonstrated by the relatively low antibody formation rate, it would also be difficult to amass a large enough patient population (or animal population for that matter) with an authentic positive antibody response. Finally, because accelerated healing would constitute an additional claim for the device that is not specifically necessary for its use, the agency did not believe this type of study was necessary as a condition of approval.

As a result, the sponsor was required to perform only the first two nonclinical studies recommended by the advisory panel. Specifically, the sponsor was required to perform:

- studies to assess the effects of rhBMP-2 on tumor promotion. These investigations included *in vitro* studies with primary tumor cell isolates. Observations from these studies could indicate a necessity to modify the device's labeling.

- studies to investigate the potential for an immune response to rhBMP-2 to interfere in embryonic development in rabbits. Observations from these studies could indicate a necessity to create a pregnancy monitoring database or modify the device's labeling.

Furthermore, the sponsor also agreed to develop the following assays in order to better define the immunological response to rhBMP-2 :

- development and validation of a new ELISA for rhBMP-2 that is capable of detecting all antibody isotypes; and

- development and validation of an assay which is capable of detecting neutralizing antibodies to rhBMP-2.

The sponsor was required to submit reports on three additional assays, *i.e.*, silver stained SDS-PAGE, Ellman's test and glycoform analysis, to be added to the release specifications for the rhBMP-2 device component.

Finally, the sponsor was required to maintain a plan for surgeon training in the use of INFUSE Bone Graft that included information related to potential benefits and risks present in the exposure to osteogenic factors, *e.g.*, immune response issues for the general population, immune response issues specific to women of child-bearing potential and issues related to tumor formation or promotion. Any changes to the type(s) of instructional information to be provided, including descriptions of "hands-on" sessions, would need to be outlined in an annual report.

CDRH worked with the sponsor to finalize product labeling and the requirements of the post approval studies outlined above. Inspections of the sponsor's manufacturing facilities were completed and found to be in compliance with the Quality System regulation.

FDA issued an approval order on April 30, 2004.

XIV. APPROVAL SPECIFICATIONS

Directions for Use: See product labeling

Hazard to Health from Use of the Device: See Indications, Contraindications, Warnings, and Precautions, and Adverse Reactions in the labeling.

Post Approval Requirements and Restrictions: See the Approval Order.